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APPLICATION NUMBER: ***60/524,645***
FILING DATE: ***November 25, 2003***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. R. Grant

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Certifying Officer

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PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
Elzbieta David C.	MIETKIEWSKA TAYLOR	Saskatoon, Saskatchewan, Canada Saskatoon, Saskatchewan, Canada

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

NASTURTIUM FATTY ACID ELONGASE (FAE) GENE AND ITS USE IN INCREASING ERUCIC ACID CONTENT

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 32
☑ Drawing(s) *Number of Sheets* 7
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☑ Other (specify)

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Respectfully submitted,

SIGNATURE

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TELEPHONE 613-237-5160

Date November 25, 2003

REGISTRATION NO. 50,180 *(if appropriate)*

Docket Number: PAT 989P-2

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Docket Number PAT 989P-2

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
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Number 1 of 1

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Complete if Known	
Application Number	
Filing Date	
First Named Inventor	Elzbieta MIETKIEWSKA et al.
Examiner Name	
Art Unit	
Attorney Docket No.	PAT 989P-2

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1003	530	2003	265	Plant filing fee	
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1203	290	2203	145	Multiple dependent claim, if not paid
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1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
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1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
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1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
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1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
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SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	David L. Conf	Registration No. (Attorney/Agent)	50,180	Telephone	613-237-5160
Signature				Date	November 25, 2003

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Nasturtium Fatty Acid Elongase *(FAE)* gene and its use in increasing erucic acid content.

Background to the invention

Very long chain fatty acids (VLCFAs) with 20 carbons or more are widely distributed in nature. In plants they are mainly found in epicuticular waxes and in the seed oils of a number of plant species, including members of the *Brassicaceae, Limnantheceae, Simmondsia* and *Tropaeolaceae*. A strategic goal in oilseed modification is to genetically manipulate high erucic acid (HEA) germplasm of the *Brassicaceae* to increase the content of erucic acid (22:1 Δ13) and other strategic VLCFAs in the seed oil for industrial niche market needs. Erucic acid and its derivatives are feedstocks in manufacturing slip-promoting agents, surfactants, plasticizers, nylon 1313, and surface coatings and more than 1000 patents have been issued. The current market for high erucate oils exceeds $120 million U.S./annum. Worldwide erucic acid demand is predicted to increase from about 40 million pounds (M pds) in 1990 to about 80 M pds by the year 2010. Similarly, demand for the derivative, behenic acid, is predicted to triple to about 102 M pds by 2010. In recent years, production has increased to meet market needs and high erucic acreage in western Canada is currently at a record high. A *Brassica* cultivar containing erucic acid levels approaching 80% would significantly reduce the cost of producing erucic acid and its derivatives and could meet the forecast demand for erucic and behenic acids as renewable, environmentally-friendly industrial feedstocks.

VLCFAs are synthesized outside the plastid by a membrane bound fatty acid elongation complex (elongase) using acyl-CoA substrates. The first reaction of elongation involves condensation of malonyl-CoA with a long chain substrate producing a 3-ketoacyl-CoA. Subsequent reactions are reduction of 3-hydroxyacyl-CoA, dehydration to an enoyl-CoA, followed by a second reduction to form the elongated acyl-CoA. The 3-ketoacyl-CoA synthase (KCS) catalyzing the condensation reaction plays a key role in determining the chain length of fatty acid products found in seed oils and is the rate-limiting enzyme for seed VLCFA production. The composition of the fatty acyl-CoA pool available for elongation and the presence and size of the neutral lipid sink are additional important factors influencing the types and levels of VLCFAs made in particular cells.

Our knowledge of the mechanism of elongation and properties of FAE1 and other elongase condensing enzymes is, in part, limited by their membrane-bound nature: as such they are more difficult to isolate and characterize than soluble condensing enzymes. The genes encoding FAE1 and its homologs have been cloned from *Arabidopsis thaliana* and from *Brassica napus* (two homologous sequences, Bn-FAE1.1 and Bn-FAE 1.2).

Site-directed mutagenesis experiments have been carried out on the *Arabidopsis* FAE1 to decipher the importance of cysteine and histidine as residues in condensing enzyme catalysis. Results have shown that cysteine[223] and four histidine residues are essential for the enzyme activity.

In this work, we selected *Tropaeolum majus*, garden nasturtium, as a source of the elongase involved in VLCFA synthesis based on the fact that this plant is capable of producing significant amounts of erucic acid (70-75 % of total fatty acid) and accumulates trierucin as the predominant triacylglycerol (TAG) in its seed oil. Here, we report the isolation of a nasturtium *FAE* gene and demonstrate the involvement of its encoded protein in the elongation of saturated and especially monounsaturated fatty acids.

This invention relates to a nasturtium cDNA encoding an "elongase" (condensing enzyme) with a high specificity for eicosenoyl moieties which can be utilized to engineer seed oil crops for production of high erucic acid oils.

There is interest in modifying the seed oil fatty acid composition and content of oilseeds by molecular genetic means to provide a dependable source of Super High Erucic Acid Rapeseed (SHEAR) oil for use as an industrial feedstock.

Nonetheless, to date, increases in the content of some strategic fatty acids have been achieved by introduction of various fatty acid biosynthesis genes in oilseeds. Some examples include:

Expression of a medium chain fatty acid thioesterase from California Bay, in Brassicaceae to increase the lauric acid content. (Calgene)

Expression of an anti-sense construct to the Δ9 desaturase in Brassicaceae to increase the stearic acid content. (Calgene)

Increased proportions of oleic acid by co-suppression using constructs encoding plant microsomal desaturases. (DuPont/Cargill)

Expression of a *Jojoba* "elongase"3-keto-acyl-CoA synthase in low erucic acid (canola) *B. napus* cultivars to increase the level of erucic acid; the effect following expression in high erucic acid cultivars was negligible (Calgene, Lassner et al., 1996).

However, there has not been an elongase gene identified or characterized as encoding an FAE with the ability to produce 22:1 beyond the level already existing in HEAR*B. napus* cultivars.

We considered that the isolated FAE "elongase" homolog from *Tropaeolum majus* (garden nasturtium) with GenBank Accession No. AY082610 (published on March 6th, 2002), could be used to engineer plants to produce seed oils highly enriched in erucic acid. We found that

to date, this is the first "elongase" transgene experiment to result in a 5-6-fold increase in the proportions erucic acid in plants.

To our knowledge the nearest art relates to an elongase gene (*FAE1*) from *Arabidopsis* which was cloned and published as: James, D.W. Jr., Lim, E., Keller, J., Plooy, I., Ralston,E. and Dooner, H.K. (1995) Directed tagging of the Arabidopsis FATTY ACID ELONGATION1 (FAE1) gene with the maize transposon activator. *The Plant Cell* 7: 309-319 (1995).

The reader is also referred to sequences 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 35, 37, 39, 41 from Jaworski, J.G. and Blacklock, B.J. Patent Application WO0194565 as well as sequences 19, 20, 21, 22, 23 from Kunst and Clemens, Regulation of embryonic transcription in plants. Patent Application WO0111061; 15-FEB-2001; University of British Columbia (CA).

Summary of the Invention

The invention relates to an expression vector for transforming a plant cell, said expression vector comprising a gene coding for a nasturtium fatty acid elongase gene in reading frame alignment with a promoter capable of increasing expression of said gene, when said transformed plant cell is in a seed, sufficient to increase in proportion of very long chain monounsaturated fatty acid when compared with a control plant cell. The invention also relates to a plant cell comprising a heterologous gene coding for a nasturtium fatty acid elongase gene or allelic variant thereof, said plant cell being capable of producing an increase, preferably at least a 10% increase, in proportion of a very long chain monounsaturated fatty acid when compared with a control plant cell lacking said heterologous gene. The increase can be larger, e.g. up to about five or six-fold. The invention also relates to seeds and plants comprising such plant cells and the use of such vectors to produce such plant cells, seeds and plants. The plant preferably is a dicotyledon, especially a member of the *Brassicaceae*, *Limnanthaceae*, *Tropaeolaceae* or *Simmondsia*.

The fatty acid elongase (often designated FAE or 3-ketoacyl-CoA synthase (KCS)) is a condensing enzyme and is the first component of the elongation complex involved in synthesis of erucic acid (22:1) in seeds of *Tropaeolum majus* (garden nasturtium). Using a degenerate primer approach a cDNA of a putative embryo *FAE* was obtained showing high homology to known plant elongases. This cDNA contains a 1512-nucleotide open reading frame (ORF) that encodes a protein of 504 amino acids. A genomic clone of the nasturtium *FAE* was isolated and sequence analyses indicated the absence of introns. Northern hybridization showed the expression of this nasturtium *FAE* gene to be restricted to the embryo. Southern hybridization revealed the nasturtium 3-ketoacyl-CoA synthase to be encoded by a small mulitigene family. To establish the function of the elongase homolog, the cDNA was introduced into two different heterologous chromosomal backgrounds, *Arabidopsis* (*A. thaliana*) and tobacco (*Nicotiana tabacum*), under the

control of a seed-specific (napin) promoter and the tandem 35S promoter, respectively. Seed-specific expression resulted in up to a 6-fold increase in erucic acid proportions in *Arabidopsis* seed oil. Constitutive expression in transgenic tobacco tissue resulted in increased proportions of very long chain saturated fatty acids. These results indicate that the nasturtium *FAE* gene encodes a condensing enzyme involved in the biosynthesis of very-long-chain fatty acids, utilizing monounsaturated and saturated acyl substrates. It shows utility for directing or engineering increased synthesis of erucic acid in other plants.

Brief description of the Figures

Figure 1. Substrate specificity of elongase(s) from mid-developing nasturtium (*T. majus*) embryos. 200 μg of protein from a 15,000 *x g* particulate fraction was used in the elongase assay. Reaction conditions were as described in Materials and Methods. Results represent the average of three replicates. For each [1-^{14}C]-acyl-CoA substrate, the relative proportional distribution of radiolabeled fatty acid elongation product(s) is(are) demarcated.

Figure 2 A: Comparison of the amino acid sequences of the nasturtium FAE homolog (NasFAE; accession no. AY0826190) with fatty acid elongase1 (FAE1) and related 3-ketoacyl-CoA synthases from other plant species. The alignment contains the sequences of the corn (ZeaFAE), *Limnanthes* (LimFAE), jojoba (SimFAE), *Arabidopis* (AraFAE) *Brassica* (BraFAE) and two *Arabidopis* 3-ketoacyl-CoA synthases associated with wax synthesis (AraKCS, AraCUT). The GenBank Accession numbers for the sequences shown are AJ292770 (ZeaFAE), AF247134 (LimFAE), U37088 (SimFAE), AF053345 (AraKCS), AF129511 (AraACUT), U29142 (AraFAE), AF009563 (BraFAE). Conserved cysteine and histidine residues are labeled with diamonds and triangles, respectively. Tyrosine at position 429 in the nasturtium FAE polypeptide is indicated by an asterisk.

B: Dendrogram of the 3-ketoacyl-CoA synthase gene family based on the amino acid sequences. The alignment was carried out by the Clustal W method using Lasergene analysis software (DNAStar, Madison, WI) GenBank accession numbers: AF247134 (LimFAE), U37088 (SimFAE), AY082610 (NasFAE), AJ292770 (ZeaFAE), AF053345 (AraKCS), U29142 (AraFAE), AF009563 (BraFAE), AF129511 (AraCUT).

Figure 3. Hydropathy analysis of *T. majus* FAE. **A:** Hydropathy plot of FAE indicating the presence of several hydrophobic regions. **B:** Schematic representation of the putative transmembrane domains of *T. majus* FAE amino-acid sequence as predicted by TMAP analysis (Persson and Argos 1994). Numbers shown in the boxes correspond to the residues of each domain in FAE.

Figure 4. Northern and Southern analyses of *T. majus FAE*.

A: Northern analysis of *FAE* gene expression in *T. majus*. Total RNA was isolated from roots (RT), leaves (LF), petals (PL) and embryos (EO). **B:** Southern blot analysis of the *FAE* gene in *T. majus*. Genomic DNA was digested with restriction enzymes: *Eco*RI (lane 1), *Acc*I (lane 2), *Nco*I (lane 3) and *Hind*III (lane 4).

Figure 5. A. Proportions of 20:1 Δ11 and 22:1 Δ13 in seed oils from non-transformed *A. thaliana* ecotype Wassilewskija (WS-Con), two plasmid only transgenic control lines (RD1- and RD-15), and the eighteen best *A. thaliana* T_2 transgenic lines expressing the *T. majus FAE* gene under control of the napin promoter. **B.** Proportions of 18:0, 20:0, 22:0 and 24:0 in seed oils from non-transformed *A. thaliana* ecotype Wassilewskija (WS-Con), two plasmid only transgenic control lines (RD1- and RD-15), and the eighteen best *A. thaliana* T_2 transgenic lines expressing the *T. majus FAE* gene under control of the napin promoter. The values are the average ± SD of three determinations performed on 200-seed lots.

Detailed Description of the Invention

Example 1

Plant materials

All experimental lines propagated in the greenhouse were grown at the Kristjanson Biotechnology Complex greenhouses, Saskatoon, under natural light conditions supplemented with high-pressure sodium lamps with a 16 h photoperiod (16 h of light and 8 h of darkness) at 22°C and a relative humidity of 25 to 30%. *Tropaeolum majus* plants (cultivar Dwarf Cherry Rose) were grown in the greenhouse and flowers were hand-pollinated. Seeds at various stages of development were harvested, their seed-coats were removed and embryos were frozen in liquid nitrogen and stored at -80°C. Tobacco plants were grown under sterile conditions on MS medium (Murashige and Skoog, 1962) as well as under normal greenhouse conditions. *Arabidopsis* plants were grown in a growth chamber at 22°C with photoperiod of 16 h light (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 8 h dark.

Nasturtium embryo protein preparations and elongase assays

A 5-15,000 *x g* particulate fraction enriched in elongase activity was isolated essentially according to Löhden and Frentzen (1992). Briefly, embryos (2-3 grams) were ground in a mortar under liquid nitrogen and then 10 ml of IB buffer (80 mM HEPES pH 7.2, containing 2 mM DTT, 320 mM sucrose and 5% PVPP) per g fresh weight was added. The homogenate was filtered through Miracloth and spun for 5 min at 5, 000 *x g* in a Sorvall refrigerated centrifuge at 5 °C, the supernatant retained and re-centrifuged at 15, 000 *x g* for 25 min. The resulting pellet was resuspended in 80 mM HEPES containing 20% glycerol and 2 mM DTT. The concentration of protein was determined by the method of Bradford (1976). This subcellular fraction was either used directly to determine enzymatic activities or stored at -80°C until used.

The 15,000 *x g* particulate preparation was used to perform elongation assays as described by Taylor et al., (1992a & b) with the following modifications: The assay mixture consisted of 80 mM HEPES-NaOH, pH 7.2 containing 0.75 mM ATP, 10 µM CoA-SH, 0.5 mM NADH, 0.5 mM NADPH, 2 mM $MgCl_2$, 200 µM malonyl-CoA, 18 µM [1-^{14}C] acyl-CoA (0.37 GBq · mol^{-1}) and nasturtium protein in a final volume of 500 µL. The reaction was started by the addition of 200 µmg of protein and incubated in a shaking water-bath at 30°C, 100 rpm for 0.5 h. [1-^{14}C]-Radiolabeled acyl-CoAs were synthesized from the corresponding free fatty acids as described previously by Taylor et al., (1990). Elongase reaction assays were stopped with 3 mL of 100gL^{-1} KOH in methanol. Fatty acid methyl esters (FAMEs) were prepared and quantified by radio-HPLC as described previously (Taylor et al., 1992b).

Lipid analyses

The total fatty acid content and acyl composition of tobacco plant lipids and *Arabidopsis* seed oils was determined by GC of the FAMEs with 17:0 FAME as an internal standard as described previously (Zou et al., 1997; Katavic et al., 2001; Taylor et al., 2001)

Isolation of FAE cDNA by a degenerate primers approach

Degenerate primers were designed for amino acid sequences conserved among *Arabidopsis thaliana* KCS1 (AF053345), *Brassica napus* FAE1 (AF009563), *Limnanthes douglasii* FAE (AF247134) and *Simmondsia chinensis* FAE (U37088). Single-stranded cDNA template for reverse transcriptase-PCR was synthesized at 42°C from embryo poly (A) RNA with PowerScript™ (Clontech). A 50 µL PCR reaction contained single-stranded cDNA derived from 40 ng of poly (A) RNA, 20 pM of each primer: F-forward TCT(A/T)GG(A/T)GG(C/A)ATGGGTTG [LGGMGC], F-reverse T(G/A)TA(T/C)GC(C/T)A(A/G)CTC(A/G)TACC [WYELAY] and 2.5 U of Taq DNA Polymerase (Amersham) under standard conditions. An internal part of the elongase sequence was

amplified in a thermolcycler during 30 cycles of the following program: 94°C for 30 sec, 48°C for 30 sec and 72°C for 1 min. The sequence of a 650-bp PCR product was used to design a primer to amplify the 5' and 3' ends of the cDNA using the SMART™ RACE cDNA Amplification Kit (CLONTECH). After assembly to determine the full length sequence of the cDNA, the open reading frame (ORF) was amplified using the primers P- forward ACCATGTCAGGAACAAAAGC and PR-reverse TTAATTTAATGGAACCTCAACCG, and subsequently cloned into the pYES2 expression vector (Invitrogen).

cDNA library construction

To construct the nasturtium developing cDNA library, immature seeds were collected 17 days after pollination. Total RNA was extracted from embryos according to Lindstrom and Vodkin (1991), then poly (A) RNA was isolated using Dynabeads Oligo$(dT)_{25}$ (DYNAL). Copy DNA synthesis was performed on 1 μg of poly (A) RNA using SMART PCR cDNA Synthesis Kit (Clontech) according to manufacturer's protocol. The cDNA population was then subtracted with 12S and 2S seed storage protein cDNA clones using PCR-Select cDNA Subtraction Kit (Clontech). The subtracted embryo cDNA population was cloned and then sequenced as described by Jako et al. (2002).

Sequence handling

Sequence analyses were performed using Lasergene software (DNAStar). Sequence similarity searches and other analyses were performed using BLASTN, BLASTX (Altschul et al., 1990) and PSORT (Nakai and Kanehisa, 1992) programs.

Site directed mutagenesis of FAE

A site-directed mutagenesis experiment was performed essentially as described previously (Katavic et al., 2002). The desired mutation (tyrosine at position 429 is replaced with histidine) was introduced into the FAE coding region by polymerase chain reaction using primers F1-forward TCGAGGATGTCGCTTCACCGATTTGGAAACAC and R1-reverse GTTTCCAAATCGGTGAAGCGACATCCTCGATGG. Primers were complementary to the opposite strands of pYES2.1/V5-His-TOPO containing the nasturtium *FAE* gene.

Northern analysis

Total RNA from nasturtium plant material was isolated according to Lindstrom and Vodkin (1991). 20 microgram of RNA was fractionated on a 1.4% formaldehyde-agarose gel and the gels were then stained with ethidium bromide to ensure that all lanes had been loaded equally (Sambrook et al., 1989). The RNA was subsequently transferred to Hybond N^+ membrane and hybridized with the ^{32}P labeled *FAE* DNA probe, prepared using the Random Primers DNA labeling kit (Gibco-BRL, Cleveland). Membranes were hybridized at 60°C overnight.

Plant transformation vectors

The coding regions of the nasturtium *FAE* (natural and mutated versions named SF and SMF, respectively) were amplified by polymerase chain reaction with primers BF-forward: taggatccATGTCAGGAACAAAAGC (lower case indicates the restriction site for BamHI); and SR-reverse tagagctcTTAATTTAATGGAACCTCAACC (lower case indicates the restriction site for *Sac*I enzyme) and subsequently cloned as a *Bam*HI and *Sac*I fragment behind the constitutive 35S promoter in binary vector pBI121 (CLONTECH).

The coding region of the nasturtium *FAE* was cloned behind the seed-specific napin promoter as follows: A *Bam*HI site was introduced in front of the start codon and behind the stop codon of *FAE* by PCR with primers BF (as above) and BR-reverse: taggatccTTAATTTAATGGAACCTCAACC (lower case indicates the restriction site for *Bam*HI). The *B. napus* napin promoter was cloned in *Hind*III/*Xba*I sites of the pUC19 (Fermentas) and the nos terminator was introduced as an *Eco*RI/*Bam*HI fragment. The resulting vector was named pDH1. The napin promoter/nos terminator cassette was excised by *Hind*III/*Eco*RI digestion and subsequently cloned into the respective sites of pRD400 (Clontech) resulting in pVK1. The coding region of *FAE* was then cloned into the *Bam*HI site of pVK1 behind the napin promoter and the resulting vector was named NF. Sense orientation of the *FAE* coding region with respect to the promoter was confirmed by restriction analyses with *Xba*I.

The final binary vectors (SF: 35S-FAE, SMF: 35S-Mutated FAE, and NF: napin:FAE) were electroporated into *Agrobacterium tumefaciens* cells strain GV3101 containing helper plasmid pMP90. Plasmid integrity was verified by DNA sequencing following its re-isolation from *A. thumefaciens* and transformation into *E. coli.*

Plant transformation and genetic analysis

Tobacco (*Nicotiana tabacum* cv. Xanthi) was transformed using a leaf disc transformation procedure (Horsch et al., 1985). Shoots that rooted in the presence of 50 μg/mL kanamycin were considered to be transgenic. Transgenic plants were transferred to soil and grown in the greenhouse.

Arabidopis (*A. thaliana* ecotype Wassilewskija) were transformed by vacuum infiltration according to the method of Clough and Bent (1998). Transgenic plants were selected and analyzed essentially as described by Jako et al., (2001).

Molecular analysis of transgenic plants

DNA was isolated from 2-3 g of tobacco or 150 mg of *Arabidopsis* leaf material using a urea-phenol extraction method (Chen et al., 1992) with the following minor modification: Material was frozen in liquid nitrogen and kept at -80°C until used. Extraction was performed for

15 min at room temperature and 400 mM ammonium acetate, pH 5.2 was used for the first two precipitation steps. Stable integration of the napin:*FAE*:nos cassette into the genome of transgenic plants was checked by PCR amplification on genomic DNA with NN3 and NN4 primers as described by Katavic et al., (2001).

Southern analyses were performed to further confirm and select those transformants containing single or multiple copies of the inserted fragments. 15 microgram of tobacco or 1 microgram of *Arabidopsis* genomic DNA was digested with the restriction enzyme *SacI*, and the resulting fragments were separated on a 0.9% (w/v) agarose gel, transferred to Hybond N^+ nylon membrane (Amersham) via an alkali blotting protocol. A 1.5 Kbp probe containing the coding sequence of *FAE* was generated by polymerase chain reaction (PCR) using primers: P1-forward ATGTCAGGAACAAAAGC and P2-reverse TAATTTAATGGAACCTCAACCG and subsequently radioactively labeled with ^{32}P as described above. Hybridization was performed at 65°C. The filters were washed once in 1x SSPE, 0.1% SDS for 15 min and in 0.1x SSPE, 0.1% SDS for 5-10 min at the temperature of hybridization. The blots were developed by exposure to X-OMAT-AR film (Kodak, Rochester, NY).

To estimate the number of *FAE* isoforms in the *T. majus* genome, 15 microgram of genomic DNA was digested with restriction enzymes: *Eco*RI, *Acc*I, *Nco*I and *Hind*III. Blotting and hybridization conditions were essentially as above except that filters were washed at low stringency with 1x SSPE, 0.1% SDS for 15 min, autoradiographed and then washed subsequently with 0.1x SSPE, 0.1% SDS, and re-exposed.

Example 2

Acyl Composition of *T. majus* cv Dwarf Cherry Rose

The acyl composition of the TAG fraction of this cultivar was typical in thatit had highly enriched proportions of very long chain monounsaturated fatty acids (VLCMFAs), particularly 22:1 (77.5%) and 20:1 (16.0%) with a trace of 24:1 (1.5%), and a low proportion of total C_{18} fatty acids (2.5%), primarily 18:1 (2.4%). The predominant TAG species were trierucin followed by 22:1/20:1/22:1 (Taylor et al., 1992a).

Example 3

Substrate specificity of nasturtium embryo elongases *in vitro*

Although there has been considerable debate regarding the acyl substrate for elongase activity in developing oilseeds, recent studies of developing seeds of *B. napus* have revealed the presence of two types of elongation activity *in vitro*: an acyl-CoA-dependent activity, and an ATP-dependent activity which apparently utilizes an endogenous acyl primer. A 15,000 *x g* particulate fraction was isolated from nasturtium embryos collected at mid-development (at 14-17

days after pollination), the stage which exhibited the highest enrichment in acyl-CoA-dependent elongase activity.

It has been shown that while ATP is necessary for acyl-CoA-dependent elongation *in vitro*, too high a concentration of ATP strongly inhibited elongase activity. In addition, elongase enzyme activity has been reported to be stimulated by the presence of 10 μM CoASH. In order to optimize reaction conditions, we assessed the roles of these two co-factors. Elongase activity was measured *in vitro* in the 15,000 *x g* particulate fraction from nasturtium embryos under different ATP concentrations (0-5 mM) in the presence of 10 μM CoASH with 18 μM 1-[^{14}C]-18:1-CoA and 200 μM malonyl-CoA. The highest activity was found at a concentration of 0.75mM of ATP. Then, elongase activity was examined with range of [1-^{14}C]-acyl-CoAs substrates at an ATP concentration of 0.75mM in the presence of 10 μM CoASH.

Our results indicate that in a developing nasturtium embryo particulate fraction, acyl-CoA-dependent elongases have the capacity to elongate a wide range of saturated (C_{16}-C_{20}) and monounsaturated (C_{18} and C_{20}) fatty acyl moieties (Fig. 1). Of the [1-^{14} C]-labeled acyl-CoA series (16:0-CoA, 18:0-CoA, 18:1-CoA, 20:0-CoA, 20:1-CoA, 22:1-CoA), tested *in vitro*, elongase(s) from mid-developing nasturtium embryos exhibited the highest activity with 18:1-CoA and 20:1-CoA (102 and 95 pmol/min/mg protein, respectively). These elongase activity rates are of the same order of magnitude as that reported for acyl-CoA elongase(s) in a similar particulate fraction from developing rapeseed embryos. The particulate fraction was also able to elongate, in order of specificity, the saturated substrates 18:0-CoA, 16:0-CoA, and to a much lesser extent, 20:0-CoA. In general, regardless of the 1-[^{14}C]acyl-CoA substrate supplied *in vitro*, the major labeled fatty acyl product was the C_2 extension of its respective precursor (about 80-90%), with the next respective C_4 extension product being present in proportions of about 10-20% (Figure 1). The one critical exception to this trend was the production solely of radiolabeled erucic acid from its respective 1-[^{14}C] eicosenoyl-CoA precursor. There was no detectable elongation of 1-[^{14}C]-labeled 22:1-CoA to 24:1, even though the latter is found in trace amounts in nasturtium seed oil.

Example 4

Isolation of *T. majus FAE* homolog

Based on sequence homology among plant fatty acid elongase genes, a full-length clone was amplified by PCR using a degenerate primers approach and the sequence submitted to the GenBank (accession number AY082610; Figure 2 (A)). The nucleotide sequence had an open reading frame of 1512 bp. Subsequently, 3 partial clones of about 0.6 kb, representative of the AY083610 *FAE* clone, were found among 2,800 ESTs surveyed (about 0.1% representation) from a nasturtium embryo subtracted cDNA library.

Alignment of the amino acid sequence of the nasturtium FAE with other plant condensing enzymes revealed the presence of six conserved cysteine residues (Fig 2A.). Further sequence analysis showed that one out of the four conserved histidine residues suggested to be important for *Arabidopsis* FAE1 activity, was substituted with tyrosine in the *T. majus* FAE polypeptide.

An analysis of the nucleotide sequence of the corresponding nasturtium *FAE* genomic clone revealed the absence of intron sequences. A similar absence of introns was observed in homologs from *A. thaliana FAE1*, rapeseed CE7 and CE8 and high and low erucic lines of *B. oleracea, B. rapa*, canola *B. napus* cv Westar and HEAR *B. napus* cv Hero.

The *T. majus* FAE cDNA encodes a polypeptide of 504 amino acids that is most closely related to an FAE2 from roots of *Zea mays* (69 % amino acid identity) (Fig. 2 (B)). The *T. majus* FAE polypeptide also shared strong identity with FAEs from *Limnanthes douglasii* (67%) and from seeds of jojoba (*Simondsia chinensis*) (63%). Homology of the nasturtium FAE to two *Arabidopsis* 3-ketoacyl-CoA synthases AraKCS and AraCUT1) involved in cuticular wax synthesis was on the level of 57% and 53%, respectively. These homologs all exhibit the capability to elongate saturated fatty acids to produce saturated VLCFAs. The FAE1 polypeptides involved in the synthesis of VLCFAs in *Arabidopsis* and *Brassica* seeds showed approximately 52-54% identity with the *T. majus* FAE. The nasturtium FAE protein was predicted to have a theoretical pI value of 9.3 using the algorithm of Bjellqvist et al., (1993 and 1994) and a molecular mass of 56.8 kDA, which are similar to the respective values reported for the *B. napus* CE7 and CE8 FAE homologs as well as those from *B. rapa* (*campestris*) and *B. oleracea*.

A hydropathy analysis (Kyte-Doolittle) of the amino acid sequence of the *T. majus* FAE revealed several hydrophobic domains (Fig. 3A). Protein analyses with the TMAP algorithm (Person and Argos, 1994) predicted two *N*-terminal transmembrane domains, the first corresponding to amino-acid residues 35-55, and the second spanning residues 68-88 (Fig 3B). Like other elongase condensing enzymes, the *T. majus* FAE lacks *N*-terminal signal sequences typically found for plastidial or mitochondrial-targeted plant enzymes. It also lacks a KXKXX or

KKXX motif (X=any amino acid) often found at the C-terminus of proteins retained within ER membranes. Rather, it is a type IIIa protein, typically present on endoplasmic reticular membranes.

Example 5

Tissue specific expression and copy number estimate of *T. majus FAE*

Northern blot analyses were performed to investigate the expression profile of the *FAE* gene. Total RNA was isolated from different nasturtium tissues including roots, leaves, floral petals and mid-developing embryos. A strong hybridization signal with *FAE*-specific probe was observed only with RNA isolated from developing embryos (Fig. 4. A).

A Southern blot hybridization was performed with nasturtium genomic DNA digested with several restriction enzymes including *Eco*RI, *Acc*I, *Nco*I and *Hind*III. The *FAE* gene has no internal *Eco*RI, *Acc*I or *Nco*I sites, while one internal *Hind*III site exists. Autoradiography revealed the presence of one strongly-hybridizing fragment in all cases except with *Hind*III for which two strongly hybridizing fragments were evident (Fig. 4.B). In addition a minimum of 4 weakly hybridizing fragments were detected. After washing under high stringency conditions, the number of hybridizing fragments was unchanged. Thus, we have concluded that *T. majus FAE* belongs to a multigenic family consisting of 4 to 6 members. A similar multigenic family has been found for a rapeseed *FAE1* gene member.

Example 6

Heterologous expression of the *T majus* FAE in Yeast

To study the function of the protein encoded by the *T. majus* FAE, the coding region was linked to the galactose-inducible *GAL1* promoter in the expression vector pYES2 and transformed into yeast. Transgenic yeast cells harbouring the *T. majus* FAE did not show any difference in fatty acid composition in comparison to yeast cells transformed with empty vector. A similar difficulty with expression of *Limnanthes* FAE in yeast cells has been reported.

As indicated earlier, a comparison of the predicted amino acid sequence of the nasturtium *FAE* with other plant condensing enzymes (Fig 2A) showed that one of the four conserved histidine residues, known suggested to be important for *Arabidopsis* FAE1 activity, was substituted with tyrosine in the *T. majus* FAE polypeptide. To study the importance of this histidine residue for enzyme activity, we used a site directed mutagenesis approach to replace the tyrosine 429 residue with histidine. This mutated version of nasturtium FAE was expressed in yeast cells. Analyses of fatty acid composition of transformed yeast cells showed that histidine at position 429 did not restore enzyme activity. Therefore we decided to study the function of *T. majus* FAE in plant heterologous chromosomal backgrounds.

Example 7

Expression of *T. majus FAE* in tobacco plants

To establish functional identity, the cDNA for the FAE-related polypeptides was constitutively expressed in tobacco plants under the control of the tandem 35S constitutive promoter. In addition, to assess the importance of histidine residues for enzyme activity, the tyrosine at position 429 in the nasturtium FAE was replaced with histidine and subsequently used to prepare a plant transformation vector under the control of the tandem 35S promoter. Integration of the 35S/*FAE*/Nos expression cassette into tobacco plants was confirmed by PCR amplification on genomic DNA. Fatty acid composition was determined in callus, leaves and seeds of transgenic tobacco plants.

Constitutive expression of the nasturtium FAE homologue in tobacco callus resulted in an increase in proportions of VLCFAs from 3.7 % in the wild type background to as high as 8.6% (a 132% increase) in transgenic lines (Table I). In particular, the increase in proportions of saturated VLCFAs (22:0, 24:0, 26:0) was most pronounced. The fact that the synthesis of the saturated VLCFAs occurs at the expense of 16:0 and 18:0 suggests that the nasturtium FAE is able to elongate C_{16} and C_{18} fatty acids. Expression of the mutated version of the nasturtium FAE (SMF) resulted in a slight increase in the VLCFA content in tobacco callus, on average 18.5% in comparison to the wild type background. Increased proportions of VLCFAs at the expense of LCFAs was observed in leaves of transgenic tobacco plants carrying either the nasturtium FAE or its mutated form (Table II). Comparison of fatty acid composition in tobacco tissues upon expression of the nasturtium FAE and its mutated version, revealed that tyrosine at position 429 is likely important to achieve full activity of the enzyme. A decreased proportion of 18:3 in leaves of transgenic tobacco lines in comparison to the wild type (empty vector) background suggests that the "metabolic pull" of the elongation pathway may be somewhat stronger than that of the competing desaturation pathway.

Expression of nasturtium *FAE* in tobacco seeds resulted in a 50% increase in proportions of VLCFAs from 0.6% in the wild type background to 0.9% in transgenic plants (data not shown). The relatively low proportions of VLCFAs in tobacco leaves and callus (see Tables I and II) may be an indication that (i) *in vivo*, saturated fatty acids are not present at high concentrations; therefore even a 50% increase in relative proportions does not result in high levels of VLC saturated fatty acids accumulating in glycerolipids; (ii) expression of the nasturtium *FAE* when under the control of the 35S promoter is relatively weak.

Example 8

Expression of *T. majus FAE* in *Arabidopsis* seeds

Since expression of nasturtium FAE under the control of the 35S promoter did not result in a high accumulation of VLCFA in tobacco seeds we decided to study the effect of expressing it in *Arabidopsis*. Using a vacuum-infiltration method, 18 kanamycin resistant *Arabidopsis* plants were obtained. The fatty acid composition of T_2 seeds was determined. A significant increase was observed only in the content of erucic acid (22:1 c13). On average, the level of erucic acid increased up to 3.2% (a 50% increase) in transgenic seeds comparing to 2.1% in wild type background (data not shown). In the best transgenic lines, the content of erucic acid increased up to 4.0% (a 90% increase).

Since tandem 35S-driven constitutive *FAE* expression did not result in a strong increase in VLCFA proportions in tobacco and *Arabidopsis* seeds, we decided to use the seed-specific promoter napin to study *FAE* expression in an *Arabidopsis* seed background. From vacuum-infiltration experiments, 25 kanamycin-resistant T_1 plants were selected. The T_2 progeny were collected individually from each plant and the fatty acid composition determined. Significant changes in fatty acid composition in comparison to the wild type (empty vector) were found. On average, the proportion of erucic acid (22:1 Δ13) increased from 2.1% in wild type to 9.6% in T_2 transgenic seeds at the expense of 20:1 Δ11 (Table III). Eighteen of the best transgenic lines were selected to examine the range of VLCFA proportional re-distribution induced by expression of the nasturtium FAE gene (Figure 5A and B). The erucic acid content was increased by up to 6.5-fold in line NF-8. Small increases in the proportions of 24:1 Δc15 were also observed (Table III). There was also a relatively significant increase in the proportions of the saturated VLCFAs, 22:0 and 24:0, at the expense of 18:0 and 20:0. In both the case of the VLC mono-unsaturated fatty acids (Fig 5A) and the VLC saturated fatty acids (Fig 5B), the highest proportional increases in erucic and in [behenic + lignoceric] acids were generally correlated with the concomitant reduction in the proportion of their corresponding elongase primers, eicosenoic and [stearate + arachidic] acids, respectively.

Therefore, we conclude that the nasturtium FAE is able to preferentially elongate 20:1 and [18:0 + 20:0]. As would be expected, there was significant variation in the proportions of 22:1 which accumulated (Figure 5A) possibly due to positional effects from nasturtium *FAE* transgene insertion at different sites in the *Arabidopsis* genome. Similar variations were observed in the expression of a castor fatty acid hydroxylase gene (*CFAH12*) in the *Arabidopsis fad2/fae1* mutant.

In summary, we have isolated a cDNA clone from nasturtium which exhibits high

similarity to the sequences of 3-ketoacyl-CoA synthases from various plant species but has the unexpected benefit of increasing the erucic acid content by 6-fold.

Our *in vitro* findings suggest that the FAE proteins in a 15,000*x g* nasturtium particulate fraction have a broad acyl-CoA preference, with the ability to elongate both monounsaturated and saturated C_{18}-CoA and C_{20}-CoA substrates. In like manner, a partially purified jojoba FAE1 showed maximal activity with monounsaturated and saturated C_{18} and C_{20}-CoAs *in vitro*. However, it is important to note that the particulate elongation activity reported in the current study most likely represents the cumulative effect of expression of more than one member of this small gene family. Thus, from this experiment one can only conclude that the capacity to elongate both monounsaturated and saturated acyl moieties is represented in this nasturtium particulate fraction.

While genetic analyses and homology assessments might predict that the isolated nasturtium *FAE* gene might encode an enzyme which prefers to elongate saturated acyl-CoAs, the transgenic experiments in tobacco callus, tobacco leaves and in *Arabidopsis* seed, collectively confirmed that the heterologously-expressed *T. majus* FAE can elongate both monounsaturated and saturated acyl moieties. In fact, in a transgenic *Arabidopsis* background, the nasturtium FAE was much stronger than the jojoba 3-KCS in its ability to increase the level of 22:1: Introducing the jojoba cDNA into *Arabidopsis* resulted in an increase in 22:1 proportions from about 2% in the control to 4% in the transgenics. In comparison, when we introduced the *T. majus* FAE into *Arabidopsis*, the erucic acid content increased by almost an order of magnitude (6-fold) at the concomitant expense of 20:1 Δ11. The acyl composition of the transgenic *Arabidopsis* seed oil was reproportioned such that erucic and eicosenoic became about equal as the two predominant VLCFAs.

The ability of the nasturtium FAE protein to preferentially elongate 18:1-CoA and especially 20:1-CoA, is consistent the observed acyl composition of nasturtium seed oil which consists primarily of very long chain- and specifically erucoyl moieties. We postulate therefore, that whether the nasturtium *FAE* transgene results in predominantly mono-unsaturated (20:1 Δ11, 22:1 Δ13) or saturated (e.g. 20:0, 22:0) VLCFAs is more a function of the composition of the acyl-CoA pool (18:1 Δ9 and 20:1 Δ11 or 18:0 and 20:0 or, respectively) available to the condensing enzyme in the host species/target organ.

Thus, the nasturtium *FAE* homolog described herein, will have a larger engineering impact when strongly expressed in a seed-specific manner in H.E.A. *Brassicaceae* (e.g. *B. napus; B. carinata*) wherein 18:1 Δ9 [and 18:2/18:3] and 20:1 Δ11 represent a potential acyl-CoA elongation substrate pool of almost 40% over and above the existing 45% 22:1 Δ13 content.

Clearly, the current studies indicate that the nasturtium FAE expression should be combined with other genetic modifications we have made to enhance the VLCFA content of HEAR *Brassicaceae* and the proportions of erucic acid in particular, to provide an industrial feedstock oil of high value and broad applicability.

A major goal of our research is to obtain, by genetic manipulation, *Brassica napus* L. cultivars with higher levels of erucic acid (22:1) in their seed oil than in present Canadian HEA cultivars. We would like to develop a *B. napus* cultivar containing erucic acid levels above 66 mol%, ideally with more than 80% erucic acid in the seed oil. To achieve our goals we are isolating new, more efficient strategic genes for high erucic acid and preferably, trierucin, production. We selected *Tropaeolum majus*, garden nasturtium, as a source of those genes based on the fact that this plant is capable of producing significant amounts of erucic acid (70-75 % of total fatty acid) and accumulates trierucin as the predominant TAG in its seed oil. The fatty acid elongase (FAE), 3-ketoacyl-CoA synthase (KCS) is the first component of the elongation complex involved in synthesis of erucic acid (22:1) in seeds of *Tropaeolum majus* (garden nasturtium). Using a degenerate primers approach, a cDNA of an embryo *FAE* was obtained and heterologously expressed in two different plant backgrounds (*A. thaliana and N. tabacum*) under the control of a seed-specific (napin) promoter and the constitutive (tandem 35S) promoter, respectively. Seed-specific expression resulted in up to a 6-fold increase in erucic acid proportions in *Arabidopsis* seed oil. Constitutive expression in transgenic tobacco tissue resulted in increased proportions of very long chain saturated fatty acids. These results indicate that the nasturtium *FAE* gene encodes a condensing enzyme involved in the biosynthesis of very-long-chain fatty acids, utilizing monounsaturated and saturated acyl substrates. Thus, the nasturtium *FAE* homolog will have a larger engineering impact when strongly expressed in a seed-specific manner in H.E.A. *Brassicaceae* (e.g. *B. napus*) wherein 18:1 Δ9 [and 18:2/18:3] and 20:1 Δ11 represent a potential acyl-CoA elongation substrate pool of almost 40% over and above the existing 45% 22:1 Δ13 content.

In addition, heterologous expression of the nasturtium *FAE* gene in HEAR *Brassicaceae* can be combined with other genetic modifications we have made to enhance the VLCFA content of HEAR germplasm (Katavic et al., 2001; Taylor et al., 2001) and the proportions of erucic acid in particular, to provide an industrial feedstock oil of high value and broad applicability.

Expression of nasturtium *FAE* in *Arabidopsis* seeds resulted in a 6-fold increase in erucic acid content. Therefore, it is anticipated that the introduction of this gene alone, or in combination with other altered gene expression phenotypes (e.g. *FAE1* and/or *FAD2* and/or *FAD3*) into HEAR *Brassicaceae* will result in transgenic lines with improved proportions of erucic acid in the seed

oil.

Example 9

Heterologous Expression of the nasturtium *FAE* in HEAR Brassicaceae

The nasturtium *FAE* gene under the control of the strong seed-specific promoter napin, has been introduced into HEAR *Brassicaceae* (e.g. *B. napus ; B. carinata*). Considering the results obtained in *Arabidopsis* seeds, it is anticipated that there will be a strong increase in the proportion of 22:1 and saturated VLCFAs as well (by up to 10%).

Example 10

Heterologous expression in HEAR *Brassicaceae* (e.g. *B. napus* or *B. carinata*) co-transformed with the napin:*AthalFAE1*+ napin:*NastFAE* or crosses of individual *A thal FAE* transgenic lines with nasturtium *FAE* transgenic lines.

Expression of nasturtium *FAE* in HEAR *Brassicaceae* (e.g. *B. napus; B. carinata*) and the resulting proportional increase in erucic acid can be maximized by also addressing the fact that 20:1, the preferred monounsaturated substrate, is present in wild type seeds in relatively low proportions (5.5-6.5%). Therefore, for example, one can introduce the *Arabidopsis FAE1* and nasturtium *FAE* into HEAR Brassicaceae (e.g. *B. napus; B. carinata*). The first gene product should enhance conversion of 18:1 to 20:1 (Katavic et al., 2001), while the nasturtium *FAE* gene product clearly prefers to extend 20:1 to 22:1. In this manner, the maximal proportion of erucic acid is expected. To achieve this goal, one could apply a co-transformation method: The *Arabidopsis FAE* is cloned in a derivative of vector pRD400 which allows selection on kanamycin, while the nasturtium *FAE* is cloned in pCAMBIA vector which allows selection on hygromycin. Alternatively, individual transgenic lines homologous for the insertion of *A. thaliana FAE1* could be crossed with nasturtium *FAE* transgenic lines.

Table I. *Fatty acid composition of transformed tobacco calli.*

Results represent the average (± SE) of ten measurements using independent calli. Constructs: RD= Control (plasmid only) transgenic calli; SF= 35S: *T. majus FAE* transgenic calli; SMF= 35S: mutated *T. majus FAE* transgenic calli.

Construct	Fatty acid composition (% (wt/wt) of total fatty acids) [% increase]*							
	16:0	18:0	20:0	22:0	24:0	26:0	LCFA	VLCFA
RD	20.38 ± 0.12	7.99 ± 0.26	1.32 ± 0.03	0.59 ± 0.03	0.70 ± 0.03	0.89 ± 0.16	96.28 ± 0.31	3.72 ± 0.31
SF	18.01 ± 0.42	5.23 ± 0.41	1.58 ± 0.54 [19.7]	1.32 ± 0.16 [123.9]	1.93 ± 0.27 [175.7]	1.31 ± 0.20 [147.2]	91.37 ± 0.84 (0.84)	8.63 ± 0.84 [131.9]
SMF	19.48 ± 0.34	7.12 ± 0.19	1.30 ± 0.02	0.57 ± 0.03	0.73 ± 0.04	1.01 ± 0.32	95.59 ± 0.40	4.41 ± 0.40 [18.5]

* relative to value for calli from RD: the tobacco control (plasmid only) calli, set at 100%.

Table II. *Fatty acid composition of transformed tobacco leaves.*

Results represent the average (± SE) of ten measurements using leaf discs from ten independent transgenic plants. Constructs: RD= Control (plasmid only) transgenic leaves; SF= 35S: *T. majus FAE* transgenic leaves; SMF= 35S: mutated *T. majus FAE* transgenic leaves.

Construct	Fatty acid composition (% (wt/wt) of total fatty acids) [% increase]*								
	16:0	18:0	18:3	20:0	20:1c11	22:0	24:0	LCFA	VLCFA
RD	16.32 ± 0.14	3.94 ± 0.11	53.30 ± 0.72	0.53 ± 0.02	1.18 ± 0.00	0.27 ± 0.01	2.74 ± 0.09	93.77 ± 0.29	6.23 ± 0.29
SF	15.83 ± 0.14	3.35 ± 0.12	47.02 ± 0.66	0.91 ± 0.12 [71.7]	2.34 ± 0.12 [98.3]	0.42 ± 0.02 [55.6]	4.14 ± 0.15 [51.1]	88.64 ± 0.35	11.36 ± 0.35 [82.3]
SMF	15.53 ± 0.17	4.00 ± 0.12	47.25 ± 0.85	0.98 ± 0.16 [84.5]	2.61 ± 0.02 [121.2]	0.30 ± 0.01	3.24 ± 0.08 [18.2]	90.05 ± 0.28	9.95 ± 0.28 [59.7]

*relative to value for leaves from RD: the tobacco control (plasmid only) plants, set at 100%.

Table III. *Fatty acid composition of transgenic Arabidopsis T_2 seeds.*

Results represent the average ± SE of triplicate measurements using 200 seeds from 25 independent *Arabidopsis* transgenic lines.

Constructs: RD= Control (plasmid only) transgenic seeds; NF=Napin: *T. majus FAE* transgenic seeds.

Construct	Fatty acid composition (% (wt/wt) of total fatty acids) {Range} [% increase]*							
	18:0	20:1c11	22:0	22:1c13	24:0	24:1c15	LCFA	VLCFA
RD	3.72 ± 0.07 {3.35-4.03}	19.87 ± 0.26 {17.97-20.86}	0.30 ± 0.01 {0.27-0.34}	2.12 ± 0.05 {1.88-2.28}	0.11 ± 0.01 {0.09-0.15}	0.19 ± 0.01 {0.15-0.24}	70.15 ± 0.22 {69.35-71.36}	29.85 ± 0.22 {28.64-30.65}
NF	2.57 ± 0.10 {1.58-3.31}	12.78 ± 0.42 {8.87-16.85}	1.57 ± 0.12 {0.66-2.78} [423.3]	9.63 ± 0.59 {4.43-15.57} [354.2]	0.46 ± 0.03 {0.24-0.65} [318.2]	0.46 ± 0.03 {0.29-0.70} [142.1]	68.77 ± 0.47 {65.53-72.06}	31.30 ± 0.47 {27.94-34.47} [4.8]

* relative to value for seeds from RD: the *Arabidopsis* control (plasmid only) plants, set at 100%.

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Claims

1. An expression vector for transforming a plant cell, said expression vector comprising a gene coding for a nasturtium fatty acid elongase gene in reading frame alignment with a promoter capable of increasing expression of said gene, when said transformed plant cell is in a seed, sufficient to increase in proportion of very long chain monounsaturated fatty acid when compared with a control plant cell.

2. A plant cell comprising a heterologous gene coding for a nasturtium fatty acid elongase gene or allelic variant thereof, said plant cell being capable of producing an increase in proportion of a very long chain monounsaturated fatty acid when compared with a control plant cell lacking said heterologous gene.

3. A seed comprising a plurality of plant cells according to claim 2.

4. A plant comprising a plurality of plant cells according to claim 2.

5. A plant cell according to claim 2 wherein said heterologous gene codes for a 3-ketoacyl-CoA synthase.

6. A plant cell according to claim 2 wherein said very long chain monounsaturated fatty acid comprises erucic acid.

7. A plant according to claim 4 wherein said plant is a dicotyledon.

8. A plant according to claim 4 wherein said plant is a member of the *Brassicaceae.*

9. A plant according to claim 4 wherein said plant is a member of the *Limnanthaceae* or *Tropaeolaceae* or *Simmondsia*

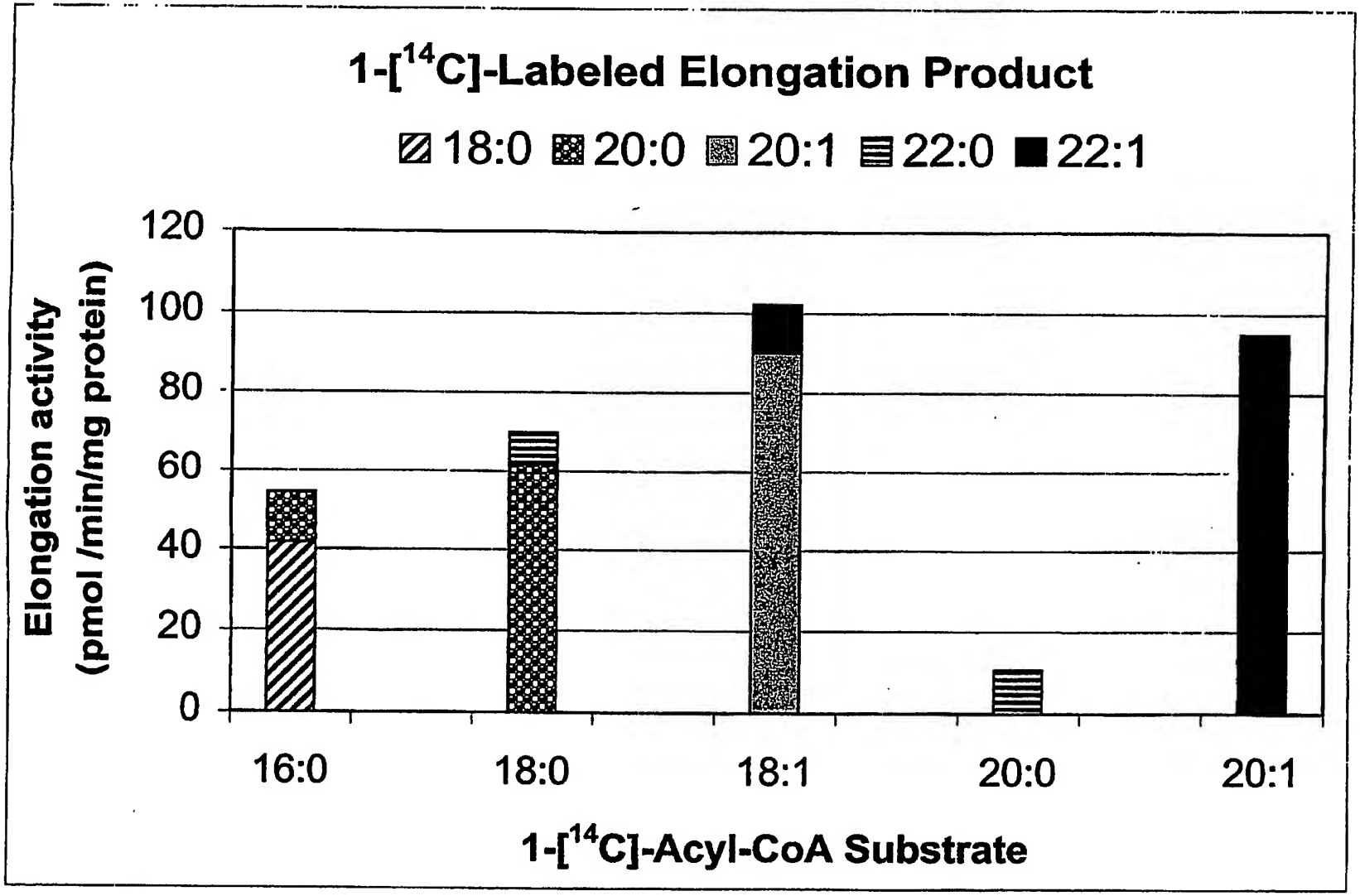
10. A plant according to claim 8 wherein said plant is of the *Brassica* genus.

11. A method for altering erucic acid content of a plant-derived oil which method comprises cultivating a plant according to claim 4 and then extracting a plant-derived oil therefrom which oil has altered erucic acid content.

12. Use of nasturtium fatty acid elongase gene for altering erucic acid content in a plant.

Abstract

This invention relates to seeds of plant, plants themselves and cells of such plants which comprise a heterologous gene coding for a nasturtium fatty acid elongase gene or allelic variant thereof, said plant or seed being capable of producing an increase in proportion of a very long chain monounsaturated fatty acid when compared with a control plant or seed lacking said heterologous gene.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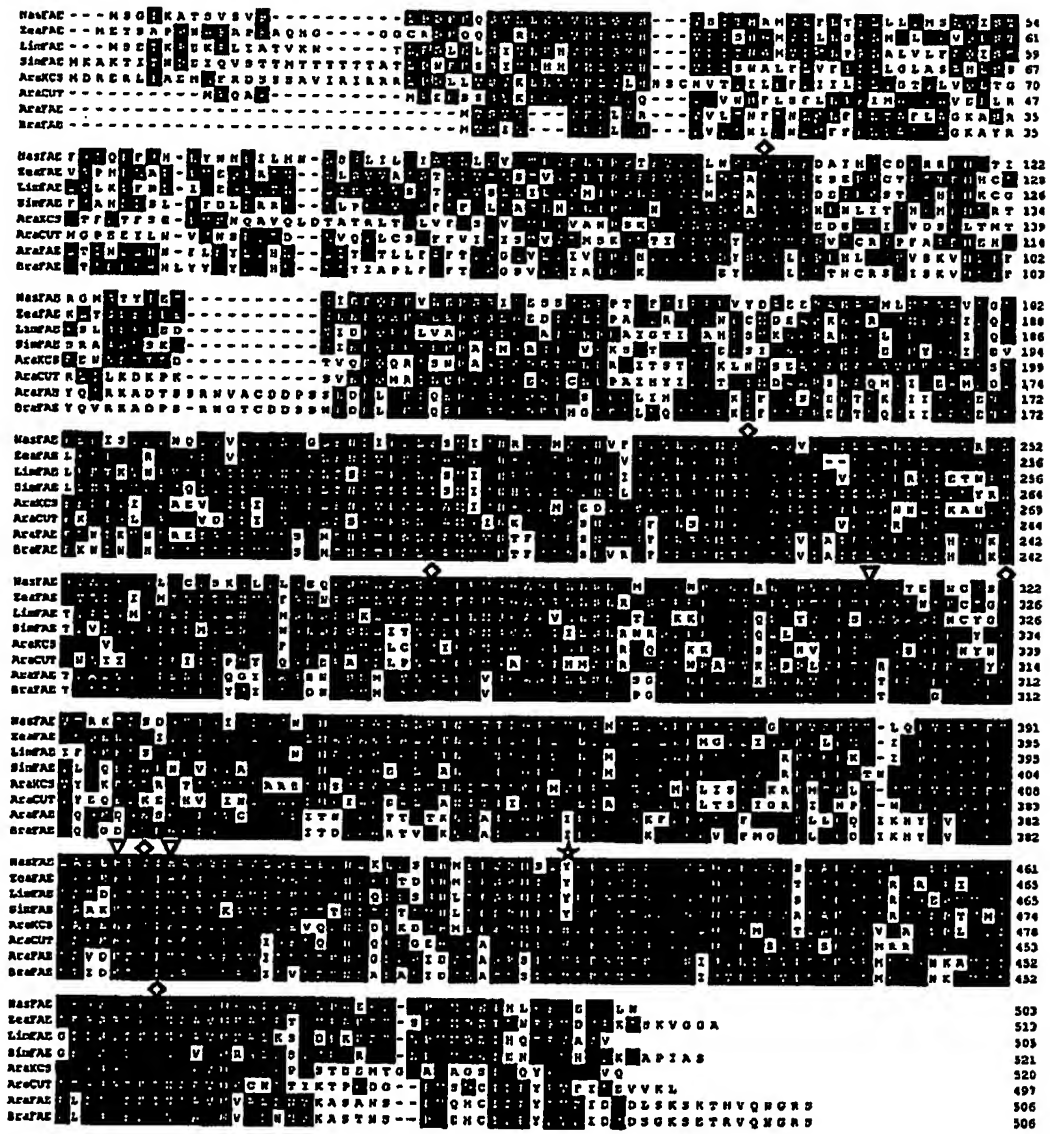


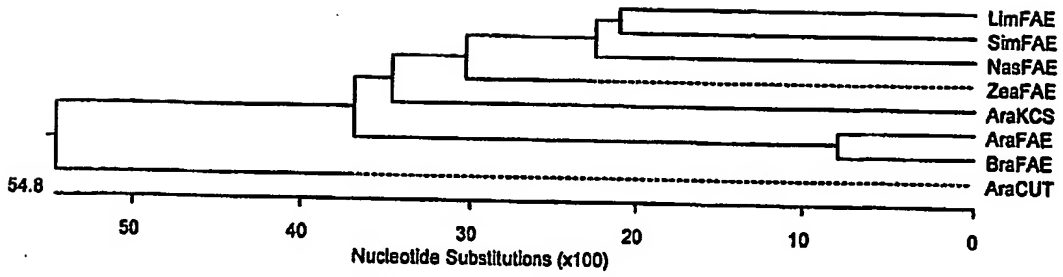


Figure 2 B. Dendrogram of the ketoacyl-CoA synthase gene family based on the amino acid sequences. The alignment was carried out by the Clustal W method using Lasergene analysis software (DNAStar, Madison, WI) GeneBank accession numbers: AF247134 (LimFAE), U37088 (SimFAE), AY082610 (NasFAE), AJ292770 (ZeaFAE), AF053345 (AraKCS), U29142 (AraFAE), AF009563 (BraFAE), AF129511 (AraACUT)

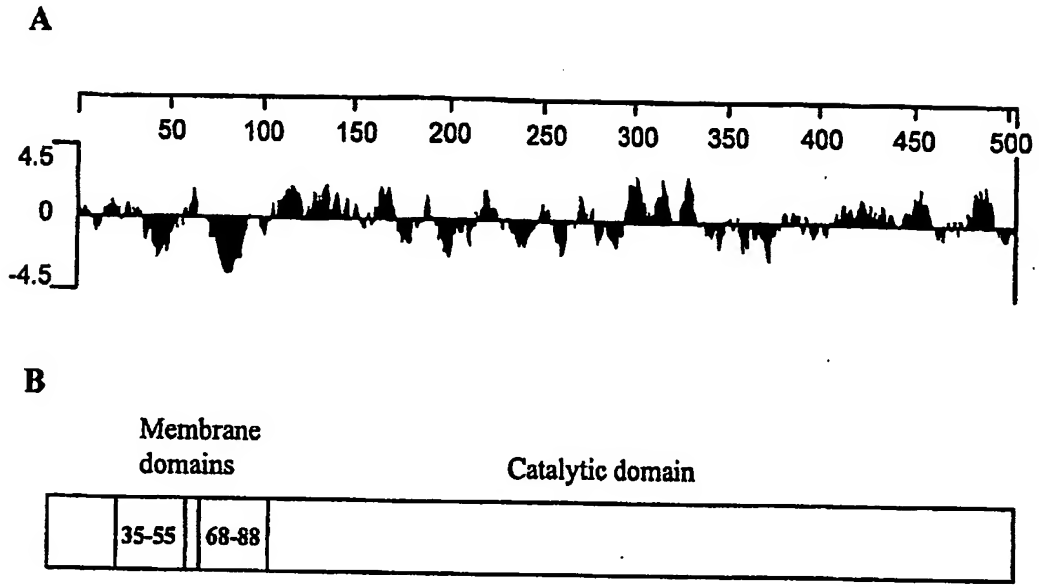
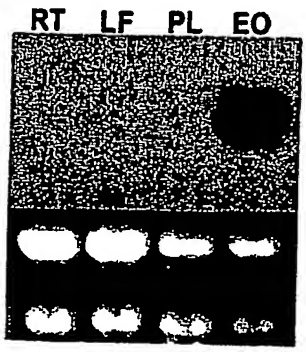


Figure 3. Hydropathy analysis of *T.majus* FAE. (A) Hydropathy plot of FAE indicating the presence of several hydrophobic regions. (B) Schematic representation of the putative transmembrane domains of *T.majus* FAE amino-acid sequence as predicted by TMAP analysis [Persson, Argos 1994]. Numbers shown in the boxes correspond to the residues of each domain in FAE.

A



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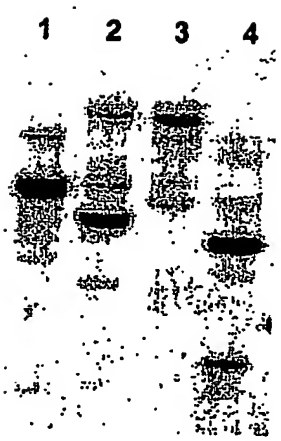
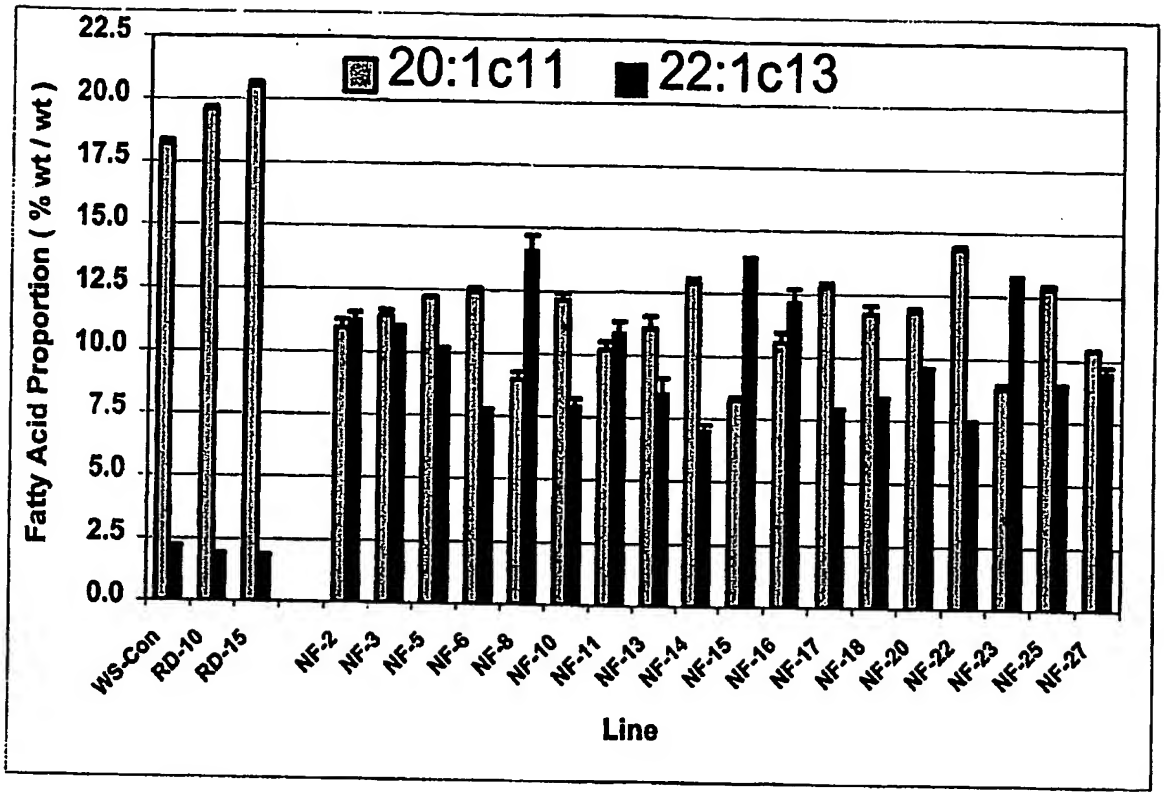


Figure 4. Northern and Southern analyses of *T.majus FAE*.

A. Northern analysis of *FAE* gene expression in *T.majus*. Total RNA was isolated from roots (RT), leaves (LF), petals (PL) and embryos (EO).

B. Southern blot analysis of the *FAE* gene in *T.majus*. Genomic DNA was digested with restriction enzymes: *Eco*RI (lane 1), *Acc*I (lane 2), *Nco*I (lane 3) and *Hind*III (lane 4).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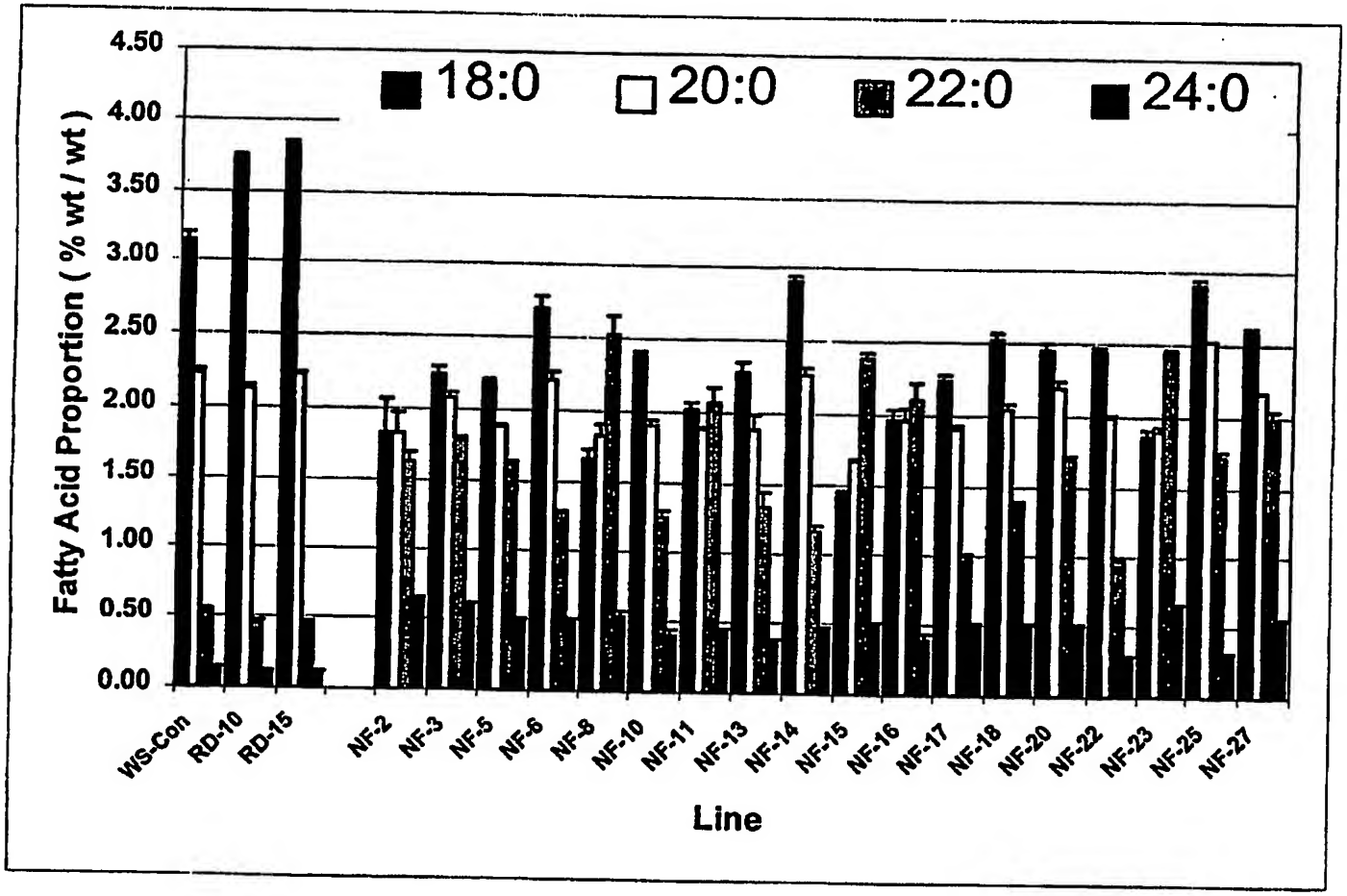
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